Carrageen

Mutagenicity Evaluation of Compound FDA 71-52 **Furcelleran (Seaweed Edible)**
Final report
4/77

Litton BIONETICS

MUTAGENICITY EVALUATION OF FDA 71-52 FURCELLERAN (SEAWEED EDIBLE)

FINAL REPORT

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MUTAGENICITY EVALUATION
OF
FDA 71-52
FURCELLERAN (SEAWEED EDIBLE)

FINAL REPORT

SUBMITTED TO

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 71-52, Furcelleran (Seaweed Edible), did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: November 24, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound FDA 71-52 Furcelleran (Seaweed Edible)

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976

2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 71-52 Furcelleran (Seaweed Edible)

2. Test solvent: Olive Oil

3. Solubility of the test compound under treatment conditions:
Suspension (insoluble)

4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination:

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	1.25	1.25
1/2 50% Survival	2.50	2.50
50% Survival	5.00	5.00

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000000001

B. TEST DATE: NOV. 13, 1976

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100	
			1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION												
SOLVENT CONTROL*	---	---	24	18	14	24	31	25	34	24	233	204
POSITIVE CONTROL**	---	---	>1000	>1000	>1000	630	>1000	>1000	899	644	986	>1000
TEST 5.00000 %	---	---	12	11	10	15	10	18	20	30	203	95
250.00000 %	---	---	20	16	14	12	25	34	43	35	96	93
12500.00000 %	---	---	14	19	24	16	27	18	35	36	101	158
2. ACTIVATION												
SOLVENT CONTROL*	MOUSE	LIVER	28	20	29	32	17	15	53	40	276	304
	RAT	LIVER	33	32	32	23	14	15	44	42	225	218
	MONKEY	LIVER	27	26	25	31	20	17	60	64	247	235
POSITIVE CONTROL***	MOUSE	LIVER	878	888	406	588	>1000	>1000	>1000	>1000	500	652
	RAT	LIVER	472	554	819	510	>1000	>1000	>1000	>1000	421	736
	MONKEY	LIVER	693	851	300	653	>1000	>1000	900	>1000	674	654
TEST 5.00%	MOUSE	LIVER	17	14	8	12	13	12	23	30	92	100
2.50%	MOUSE	LIVER	12	21	16	11	11	13	59	71	128	126
1.25%	MOUSE	LIVER	19	18	12	18	13	18	44	63	151	149
5.00%	RAT	LIVER	19	10	8	10	13	14	40	39	131	100
2.50%	RAT	LIVER	11	23	17	15	13	10	45	44	122	127
1.25%	RAT	LIVER	12	14	22	20	14	14	56	39	131	173
5.00%	MONKEY	LIVER	19	17	11	16	14	13	47	52	79	101
2.50%	MONKEY	LIVER	17	21	15	12	18	18	59	54	153	159
1.25%	MONKEY	LIVER	27	17	10	11	15	11	83	64	149	177

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES / NONACTIVATION COMPOUND 000000001

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	00000D4 ADE EX-5	00000D4 TRY EX-5	
NAN		74.62	10.24	10.77	4.18	10.69	11.13	6.91	CONTROLS
NAP		532.51	182.08	82.18	87.98	143.98	106.27	69.28	
NA1		32.99	9.45	9.31	8.87	3.15	13.57	3.90	
NA2		46.25	8.02	10.63	9.45	5.46	11.51	3.78	TEST COMPOUND
NA3		53.98	7.49	17.30	6.76	6.90	16.18	3.31	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES ICHFLO/MOUSE COMPOUND 00000001

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	134.62	15.43	11.54	3.99	21.75	9.30	3.10	
ACT	A-C	178.05	13.43	14.20	5.07	23.98	14.24	4.75	NEGATIVE CONTROLS
ACT	ALI	104.90	11.78	13.18	7.47	46.74	7.78	2.59	
ACT	ALU	117.78	12.64	20.26	6.67	33.84	7.53	2.51	
ACT	PLI	144.81	195.15	54.22	213.36	583.71	97.33	85.59	POSITIVE CONTROLS
ACT	PLU	114.22	10.16	57.82	102.63	284.16	13.76	4.23	
ACT	LII	172.84	10.97	16.84	13.12	24.62	7.87	2.62	
ACT	LI2	78.51	10.75	13.14	12.93	16.57	11.34	3.78	
ACT	LI3	134.94	13.08	17.78	9.67	25.14	17.94	5.98	TEST COMPOUND
ACT	LUI	134.17	10.55	12.29	14.48	13.24	6.95	2.32	
ACT	LU2	70.59	12.33	14.66	8.79	36.60	11.52	3.53	
ACT	LU3	85.82	14.19	13.50	9.03	38.11	11.28	3.76	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES SPRDAW/RAT COMPOUND 000000001

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	38.07	10.26	13.64	12.33	12.33	4.80	17.13	5.71	
ACT	A-C	60.38	15.10	14.41	11.48	14.94	6.36	14.16	4.72	NEGATIVE CONTROLS
ACT	ALI	61.10	10.06	15.83	9.61	7.46	14.84	13.68	4.56	
ACT	ALU	50.22	10.96	19.96	10.02	11.63	13.83	8.32	2.77	
ACT	PLI	102.12	104.55	115.35	90.18	90.18	248.92	143.57	57.65	
ACT	PLU	92.31	11.27	54.37	111.95	74.74	121.82	8.66	2.89	POSITIVE CONTROLS
ACT	LI1	29.75	9.28	10.95	8.13	10.88	8.80	19.20	6.40	
ACT	LI2	17.19	7.95	7.09	7.62	10.10	11.29	12.14	3.92	
ACT	LI3	26.71	10.77	5.14	9.31	13.81	6.87	11.55	3.54	TEST COMPOUND
ACT	LU1	42.40	9.80	4.30	7.00	8.07	9.00	12.88	4.29	
ACT	LU2	59.29	9.55	4.53	8.19	11.97	6.01	12.15	4.66	
ACT	LU3	26.96	9.22	3.75	8.28	11.55	12.85	12.53	4.18	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES RHESUS/MONKEY COMPOUND 000000001

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	77.24	8.90	6.40	6.53	5.62	10.32	5.96	
ACT	A-C	83.51	11.21	4.63	3.32	6.17	18.61	5.45	NEGATIVE CONTROLS
ACT	ALI	85.75	13.21	31.55	9.45	28.36	6.06	4.98	
ACT	ALU	84.92	10.93	36.23	10.57	24.95	13.59	6.16	
ACT	PLI	186.02	82.34	95.20	196.91	806.40	104.94	61.24	
ACT	PLU	96.52	14.26	16.84	11.15	11.87	11.24	4.23	POSITIVE CONTROLS
ACT	LII	97.81	8.13	4.88	6.63	21.24	9.14	3.29	
ACT	LI2	73.56	7.62	8.67	6.17	23.65	6.63	2.63	
ACT	LI3	79.17	9.31	7.02	5.18	15.00	8.34	4.49	TEST COMPOUND
ACT	LUI	82.74	7.00	9.93	16.53	19.04	8.11	3.70	
ACT	LU2	76.00	8.19	6.87	5.60	23.74	7.77	4.85	
ACT	LU3	87.01	8.28	5.75	7.04	19.99	6.41	3.75	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 71-52, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 71-52, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D. Date 3/31/77
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. Date 3/31/77
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

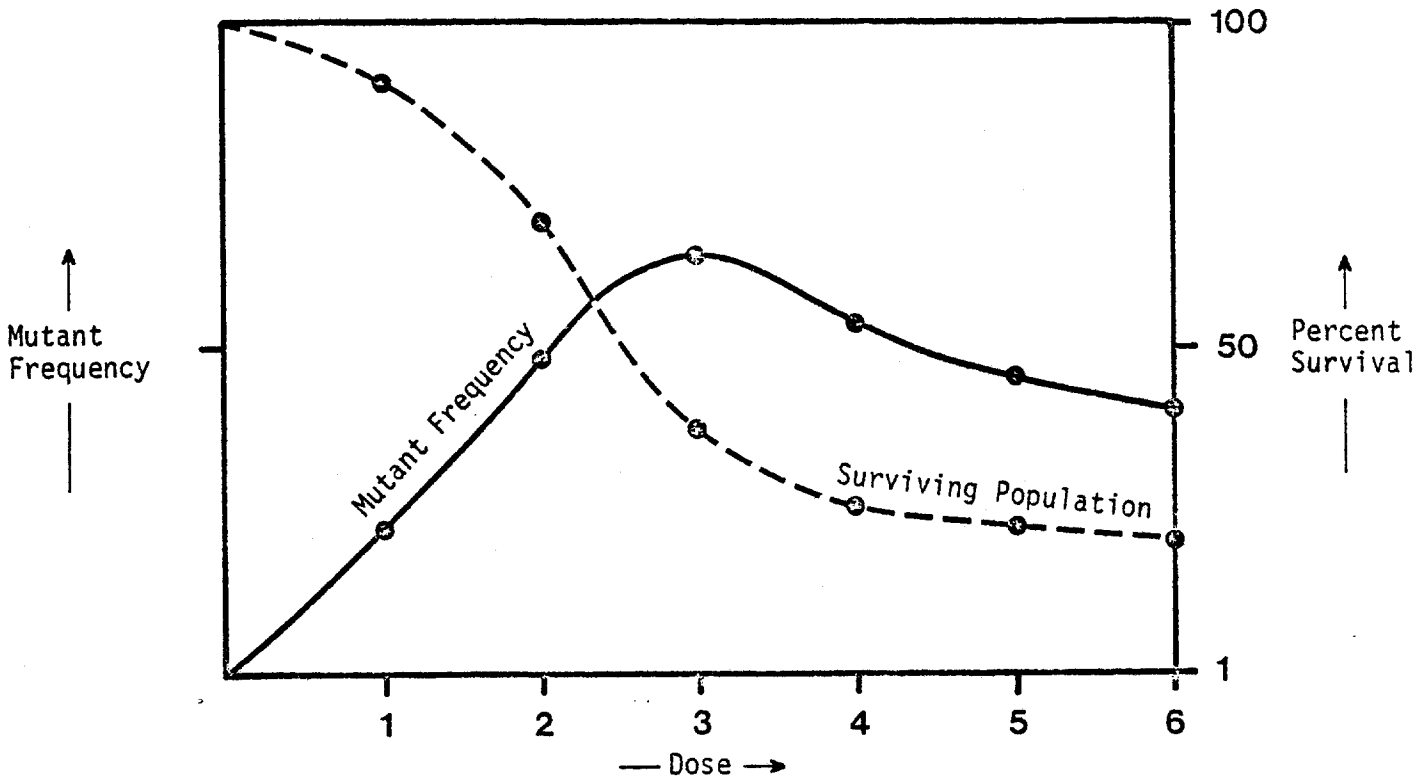
A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631703 DETECTOR TA100 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0457	0341	74.62	0
	NAP		EMS 0.066%	0486	2588	532.51	0
000000001	NA1		0005-0 PCT.	0767	0253	32.99	0
000000001	NA2		0025-1 PCT.	0534	0247	46.25	0
000000001	NA3		0125-3 PCT.	0415	0224	53.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631709 DETECTOR TA1535 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0547	0056	10.24	0
	NAP		EMS 0.2%	0558	1016	182.08	0
000000001	NA1		0005-0 PCT.	0582	0055	9.45	0
000000001	NA2		0025-1 PCT.	0636	0051	8.02	0
000000001	NA3		0125-2 PCT.	0654	0049	7.49	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631503 DETECTOR TA1537 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	1170	0126	10.77	0
	NAP		QM 13 UG/ML	1027	0844	82.18	0
000000001	NA1		0005-0 PCT.	1719	0160	9.31	0
000000001	NA2		0025-1 PCT.	1449	0154	10.63	0
000000001	NA3		0125-2 PCT.	0977	0169	17.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 631710 DETECTOR TA1538 SPECIES / PROJECT 02468

DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0407	0017	4.18	0
	NAP		NF 667 UG/ML	0391	0344	87.98	0
000000001	NA1		0005-0 PCT.	0485	0043	8.87	0
000000001	NA2		0025-1 PCT.	0487	0046	9.45	0
000000001	NA3		0125-2 PCT.	0414	0028	6.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631711 DETECTOR TA98 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0945	0101	10.69	0
	NAP		NF 667 UG/ML	0864	1244	143.98	0
000000001	NA1		0005-0 PCT.	2098	0066	3.15	0
000000001	NA2		0025-1 PCT.	1740	0095	5.46	0
000000001	NA3		0125-2 PCT.	1521	0105	6.90	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632319 DETECTOR 0000D4 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0593	0066	0041	11.13	6.91	0
	NAP		EMS 1.0 %	0638	0678	0442	106.27	69.28	0
000000001	NA1		0005-0 PCT.	0538	0073	0021	13.57	3.90	0
000000001	NA2		0025-1 PCT.	0582	0067	0022	11.51	3.78	0
000000001	NA3		0125-2 PCT.	0513	0083	0017	16.18	3.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632023 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0208	0280	134.62	0
	A-C		SOLVENT	0164	0292	178.05	0
	ALI		TISSUE	0245	0257	104.90	0
	ALU		TISSUE	0225	0265	117.78	0
	ACP	LI	DMN 90 UM/ML	0183	0265	144.81	0
	ACP	LU	DMN 90 UM/ML	0204	0233	114.22	0
000000001	ACT	LI1	0005-0 PCT.	0162	0280	172.84	3
000000001	ACT	LI2	0025-2 PCT.	0242	0190	78.51	0
000000001	ACT	LI3	0125-2 PCT.	0249	0336	134.94	0
000000001	ACT	LU1	0005-0 PCT.	0120	0161	134.17	0
000000001	ACT	LU2	0025-2 PCT.	0255	0180	70.59	0
000000001	ACT	LU3	0125-2 PCT.	0261	0224	85.82	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632106 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0350	0054	15.43	0
	A-C		SOLVENT	0469	0063	13.43	0
	ALI		TISSUE	0518	0061	11.78	0
	ALU		TISSUE	0546	0069	12.64	0
	ACP	LI	DMN 90 UM/ML	0536	1046	195.15	0
	ACP	LU	DMN 90 UM/ML	0364	0037	10.16	0
000000001	ACT	LI1	0005-0 PCT.	0401	0044	10.97	0
000000001	ACT	LI2	0025-1 PCT.	0428	0046	10.75	0
000000001	ACT	LI3	0125-2 PCT.	0413	0054	13.08	0
000000001	ACT	LU1	0005-0 PCT.	0398	0042	10.55	0
000000001	ACT	LU2	0025-1 PCT.	0438	0054	12.33	0
000000001	ACT	LU3	0125-2 PCT.	0437	0062	14.19	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631712 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1257	0145	11.54	0
	A-C		SOLVENT	1268	0180	14.20	0
	ALI		TISSUE	1055	0139	13.18	0
	ALU		TISSUE	1160	0235	20.26	0
	ACP	LI	AMQ 333 UG/ML	1741	0944	54.22	0
	ACP	LU	AMQ 333 UG/ML	0908	0525	57.82	0
000000001	ACT	LI1	0005-0 PCT.	1473	0248	16.84	0
000000001	ACT	LI2	0025-1 PCT.	1659	0218	13.14	0
000000001	ACT	LI3	0125-2 PCT.	1164	0207	17.78	0
000000001	ACT	LU1	0005-0 PCT.	1611	0198	12.29	2
000000001	ACT	LU2	0025-1 PCT.	1494	0219	14.66	2
000000001	ACT	LU3	0125-2 PCT.	1600	0216	13.50	3

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632025 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0602	0024	3.99	0
	A-C		SOLVENT	0513	0026	5.07	0
	ALI		TISSUE	0696	0052	7.47	0
	ALU		TISSUE	0480	0032	6.67	0
	ACP	LI	ANTH 67 UG/ML	0569	1214	213.36	0
	ACP	LU	ANTH 67 UG/ML	0761	0781	102.63	0
000000001	ACT	LI1	0005-0 PCT.	0442	0058	13.12	0
000000001	ACT	LI2	0025-2 PCT.	0441	0057	12.93	0
000000001	ACT	LI3	0125-2 PCT.	0424	0041	9.67	0
000000001	ACT	LU1	0005-0 PCT.	0449	0065	14.48	0
000000001	ACT	LU2	0025-2 PCT.	0455	0040	8.79	2
000000001	ACT	LU3	0125-2 PCT.	0432	0039	9.03	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631802 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1108	0241	21.75	0
	A-C		SOLVENT	1051	0252	23.98	0
	ALI		TISSUE	0875	0409	46.74	0
	ALU		TISSUE	1572	0532	33.84	0
	ACP	LI	ANTH 67 UG/ML	0356	2078	583.71	0
	ACP	LU	ANTH 67 UG/ML	0221	0628	284.16	0
000000001	ACT	LI1	0005-0 PCT.	0991	0244	24.62	0
000000001	ACT	LI2	0025-1 PCT.	1750	0290	16.57	0
000000001	ACT	LI3	0125-2 PCT.	1078	0271	25.14	0
000000001	ACT	LU1	0005-0 PCT.	1707	0226	13.24	0
000000001	ACT	LU2	0025-1 PCT.	0806	0295	36.60	0
000000001	ACT	LU3	0125-2 PCT.	0719	0274	38.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632303 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0774	0072	0024	9.30	3.10	0
	A-C		SOLVENT	0674	0096	0032	14.24	4.75	0
	ALI		TISSUE	0694	0054	0018	7.78	2.59	0
	ALU		TISSUE	0876	0066	0022	7.53	2.51	0
	ACP	LI	DMN 90 UM/ML	0562	0547	0481	97.33	85.59	0
	ACP	LU	DMN 90 UM/ML	0567	0078	0024	13.76	4.23	0
000000001	ACT	LI1	0005-0 PCT.	0724	0057	0019	7.87	2.62	0
000000001	ACT	LI2	0025-1 PCT.	0873	0099	0033	11.34	3.78	0
000000001	ACT	LI3	0125-2 PCT.	0652	0117	0039	17.94	5.98	0
000000001	ACT	LU1	0005-0 PCT.	0993	0069	0023	6.95	2.32	0
000000001	ACT	LU2	0025-1 PCT.	0851	0098	0030	11.52	3.53	0
000000001	ACT	LU3	0125-2 PCT.	0745	0084	0028	11.28	3.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632020 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0746	0284	38.07	0
	A-C		SOLVENT	0530	0320	60.38	0
	ALI		TISSUE	0617	0377	61.10	0
	ALU		TISSUE	0687	0345	50.22	0
	ACP	LI	DMN 90 UM/ML	0661	0675	102.12	0
	ACP	LU	DMN 90 UM/ML	0325	0300	92.31	0
000000001	ACT	LI1	0005-0 PCT.	0521	0155	29.75	0
000000001	ACT	LI2	0025-1 PCT.	0576	0099	17.19	0
000000001	ACT	LI3	0125-2 PCT.	0599	0160	26.71	0
000000001	ACT	LU1	0005-0 PCT.	0684	0290	42.40	0
000000001	ACT	LU2	0025-1 PCT.	0592	0351	59.29	0
000000001	ACT	LU3	0125-2 PCT.	0601	0162	26.96	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632024 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0468	0048	10.26	0
	A-C		SOLVENT	0457	0069	15.10	0
	ALI		TISSUE	0497	0050	10.06	0
	ALU		TISSUE	0429	0047	10.96	0
	ACP	LI	DMN 90 UM/ML	0571	0597	104.55	0
	ACP	LU	DMN 90 UM/ML	0426	0048	11.27	0
000000001	ACT	LI1	0005-0 PCT.	0485	0045	9.28	0
000000001	ACT	LI2	0025-1 PCT.	0528	0042	7.95	0
000000001	ACT	LI3	0125-2 PCT.	0418	0045	10.77	0
000000001	ACT	LU1	0005-0 PCT.	0459	0045	9.80	0
000000001	ACT	LU2	0025-1 PCT.	0440	0042	9.55	0
000000001	ACT	LU3	0125-2 PCT.	0434	0040	9.22	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632021 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1100	0150	13.64	0
	A-C		SOLVENT	1221	0176	14.41	0
	ALI		TISSUE	1213	0192	15.83	0
	ALU		TISSUE	1458	0291	19.96	0
	ACP	LI	AMQ 333 UG/ML	1192	1375	115.35	0
	ACP	LU	AMQ 333 UG/ML	1374	0747	54.37	0
000000001	ACT	LI1	0005-0 PCT.	0968	0106	10.95	0
000000001	ACT	LI2	0025-1 PCT.	1086	0077	7.09	0
000000001	ACT	LI3	0125-2 PCT.	1283	0066	5.14	0
000000001	ACT	LU1	0005-0 PCT.	1232	0053	4.30	0
000000001	ACT	LU2	0025-1 PCT.	1281	0058	4.53	0
000000001	ACT	LU3	0125-2 PCT.	1548	0058	3.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632009 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0576	0071	12.33	0
	A-C		SOLVENT	0636	0073	11.48	0
	ALI		TISSUE	0458	0044	9.61	0
	ALU		TISSUE	0449	0045	10.02	0
	ACP	LI	ANTH 67 UG/ML	0448	0404	90.18	0
	ACP	LU	ANTH 67 UG/ML	0452	0506	111.95	0
000000001	ACT	LI1	0005-0 PCT.	0578	0047	8.13	0
000000001	ACT	LI2	0025-1 PCT.	0617	0047	7.62	0
000000001	ACT	LI3	0125-2 PCT.	0569	0053	9.31	0
000000001	ACT	LU1	0005-0 PCT.	0643	0045	7.00	0
000000001	ACT	LU2	0025-1 PCT.	0537	0044	8.19	0
000000001	ACT	LU3	0125-2 PCT.	0580	0048	8.28	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632014 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0576	0071	12.33	0
	A-C		SOLVENT	0636	0095	14.94	0
	ALI		TISSUE	0617	0046	7.46	0
	ALU		TISSUE	0447	0052	11.63	0
	ACP	LI	ANTH 67 UG/ML	0448	0404	90.18	0
	ACP	LU	ANTH 67 UG/ML	0677	0506	74.74	0
000000001	ACT	LI1	0005-0 PCT.	0487	0053	10.88	0
000000001	ACT	LI2	0025-1 PCT.	0495	0050	10.10	0
000000001	ACT	LI3	0125-2 PCT.	0485	0067	13.81	0
000000001	ACT	LU1	0005-0 PCT.	0384	0031	8.07	0
000000001	ACT	LU2	0025-1 PCT.	0451	0054	11.97	0
000000001	ACT	LU3	0125-2 PCT.	0433	0050	11.55	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632013 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1790	0086	4.80	0
	A-C		SOLVENT	1808	0115	6.36	0
	ALI		TISSUE	0768	0114	14.84	0
	ALU		TISSUE	0716	0099	13.83	0
	ACP	LI	ANTH 67 UG/ML	0556	1384	248.92	0
	ACP	LU	ANTH 67 UG/ML	1054	1284	121.82	0
000000001	ACT	LI1	0005-0 PCT.	1295	0114	8.80	0
000000001	ACT	LI2	0025-1 PCT.	1089	0123	11.29	0
000000001	ACT	LI3	0125-2 PCT.	1761	0121	6.87	0
000000001	ACT	LU1	0005-0 PCT.	1677	0151	9.00	0
000000001	ACT	LU2	0025-1 PCT.	2179	0131	6.01	0
000000001	ACT	LU3	0125-2 PCT.	1012	0130	12.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY HACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632020 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0648	0111	0037	17.13	5.71	0
	A-C		SOLVENT	0657	0093	0031	14.16	4.72	0
	ALI		TISSUE	0614	0084	0028	13.68	4.56	0
	ALU		TISSUE	0649	0054	0018	8.32	2.77	0
	ACP	LI	DMN 90 UM/ML	0739	1061	0426	143.57	57.65	0
	ACP	LU	DMN 90 UM/ML	0658	0057	0019	8.66	2.89	0
000000001	ACT	LI1	0005-0 PCT.	0672	0129	0043	19.20	6.40	0
000000001	ACT	LI2	0025-1 PCT.	0791	0096	0031	12.14	3.92	0
000000001	ACT	LI3	0125-2 PCT.	0762	0088	0027	11.55	3.54	0
000000001	ACT	LU1	0005-0 PCT.	0652	0084	0028	12.88	4.29	0
000000001	ACT	LU2	0025-1 PCT.	0601	0073	0028	12.15	4.66	0
000000001	ACT	LU3	0125-2 PCT.	0742	0093	0031	12.53	4.18	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632126 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0312	0241	77.24	0
	A-C		SOLVENT	0279	0233	83.51	0
	ALI		TISSUE	0365	0313	85.75	0
	ALU		TISSUE	0358	0304	84.92	0
	ACP	LI	DMN 90 UM/ML	0322	0599	186.02	0
	ACP	LU	DMN 90 UM/ML	0230	0222	96.52	0
000000001	ACT	LI1	0005-0 PCT.	0320	0313	97.81	0
000000001	ACT	LI2	0025-1 PCT.	0329	0242	73.56	0
000000001	ACT	LI3	0125-2 PCT.	0360	0285	79.17	0
000000001	ACT	LU1	0005-0 PCT.	0307	0254	82.74	0
000000001	ACT	LU2	0025-1 PCT.	0300	0228	76.00	0
000000001	ACT	LU3	0125-2 PCT.	0354	0308	87.01	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632070 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0652	0058	8.90	0
	A-C		SOLVENT	0678	0076	11.21	0
	ALI		TISSUE	0636	0084	13.21	0
	ALU		TISSUE	0613	0067	10.93	0
	ACP	LI	DMN 90 UM/ML	0770	0634	82.34	0
	ACP	LU	DMN 90 UM/ML	0652	0093	14.26	0
000000001	ACT	LI1	0005-0 PCT.	0578	0047	8.13	0
000000001	ACT	LI2	0025-1 PCT.	0617	0047	7.62	0
000000001	ACT	LI3	0125-2 PCT.	0569	0053	9.31	0
000000001	ACT	LU1	0005-0 PCT.	0643	0045	7.00	0
000000001	ACT	LU2	0025-1 PCT.	0537	0044	8.19	0
000000001	ACT	LU3	0125-2 PCT.	0580	0048	8.28	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632011 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1141	0073	6.40	0
	A-C		SOLVENT	1750	0081	4.63	0
	ALI		TISSUE	0634	0200	31.55	0
	ALU		TISSUE	0530	0192	36.23	0
	ACP	LI	AMQ 333 UG/ML	0771	0734	95.20	0
	ACP	LU	AMQ 333 UG/ML	0392	0066	16.84	0
000000001	ACT	LI1	0005-0 PCT.	1127	0055	4.88	0
000000001	ACT	LI2	0025-1 PCT.	0842	0073	8.67	0
000000001	ACT	LI3	0125-3 PCT.	0926	0065	7.02	0
000000001	ACT	LU1	0005-0 PCT.	0675	0067	9.93	0
000000001	ACT	LU2	0025-1 PCT.	1237	0085	6.87	0
000000001	ACT	LU3	0125-3 PCT.	1338	0077	5.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632022 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0490	0032	6.53	0
	A-C		SOLVENT	0542	0018	3.32	0
	ALI		TISSUE	0455	0043	9.45	0
	ALU		TISSUE	0435	0046	10.57	0
	ACP	LI	ANTH 67 UG/ML	0679	1337	196.91	0
	ACP	LU	ANTH 67 UG/ML	0574	0064	11.15	0
000000001	ACT	LI1	0005-0 PCT.	0483	0032	6.63	0
000000001	ACT	LI2	0025-1 PCT.	0470	0029	6.17	0
000000001	ACT	LI3	0125-2 PCT.	0502	0026	5.18	0
000000001	ACT	LU1	0005-0 PCT.	0611	0101	16.53	0
000000001	ACT	LU2	0025-1 PCT.	0500	0028	5.60	0
000000001	ACT	LU3	0125-2 PCT.	0540	0038	7.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632012 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2027	0114	5.62	0
	A-C		SOLVENT	1134	0070	6.17	0
	ALI		TISSUE	0952	0270	28.36	0
	ALU		TISSUE	1030	0257	24.95	0
	ACP	LI	ANTH 67 UG/ML	0422	3403	806.40	0
	ACP	LU	ANTH 67 UG/ML	1070	0127	11.87	0
000000001	ACT	LI1	0005-0 PCT.	1436	0305	21.24	2
000000001	ACT	LI2	0025-1 PCT.	1277	0302	23.65	0
000000001	ACT	LI3	0125-2 PCT.	2126	0319	15.00	0
000000001	ACT	LU1	0005-0 PCT.	1544	0294	19.04	0
000000001	ACT	LU2	0025-1 PCT.	1272	0302	23.74	0
000000001	ACT	LU3	0125-2 PCT.	1631	0326	19.99	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632026 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0688	0071	0029	10.32	5.96	0
	A-C		SOLVENT	0532	0099	0041	18.61	5.45	0
	ALI		TISSUE	0743	0045	0037	6.06	4.98	0
	ALU		TISSUE	0552	0075	0034	13.59	6.16	0
	ACP	LI	DMN 90 UM/ML	0627	0658	0384	104.94	61.24	0
	ACP	LU	DMN 90 UM/ML	0685	0077	0029	11.24	4.23	0
000000001	ACT	LI1	0005-0 PCT.	0547	0050	0018	9.14	3.29	0
000000001	ACT	LI2	0025-1 PCT.	0800	0053	0021	6.63	2.63	0
000000001	ACT	LI3	0125-2 PCT.	0779	0065	0035	8.34	4.49	0
000000001	ACT	LU1	0005-0 PCT.	0703	0057	0026	8.11	3.70	0
000000001	ACT	LU2	0025-1 PCT.	0721	0056	0035	7.77	4.85	0
000000001	ACT	LU3	0125-2 PCT.	0827	0053	0031	6.41	3.75	0